PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02, 39/00, C07K 7/08 C07K 7/10, 13/00, 15/00 C07K 15/28	A1	(11) International Publication Number: WO 91/04745 (43) International Publication Date: 18 April 1991 (18.04.91)

(21) International Application Number: PCT/US90/05105

(22) International Filing Date: 13 September 1990 (13.09.90)

(30) Priority data:
413,332 27 September 1989 (27.09.89) US
571,267 23 August 1990 (23.08.90) US

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(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS FOR CELL ADHESION INHIBITION AND METHODS OF USE

(57) Abstract

Compositions that disrupt microvascular endothelial and epithelial cell tight junctions, and methods of use, are disclosed. Such compositions comprise agents that inhibit the binding to such cells of cell adhesion molecules. Such inhibitor agents include cell adhesion molecules, fragments of cell adhesion molecules that encompass a cell-binding domain such as HAV, and antibodies directed against cell adhesion molecules and fragments thereof. Also disclosed are drug delivery compositions comprising a therapeutic drug conjugated to an agent that disrupts cell tight junctions.

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COMPOSITIONS FOR CELL ADHESION INHIBITION AND METHODS OF USE

This is a continuation-in-part of United States Serial No. 07/413,332, filed September 27, 1989.

Background of the Invention

Field of the Invention

This invention relates to compositions that transiently and reversibly dissociate the blood-brain barrier. More particularly, the invention relates to compositions that dissociate tight junctions between brain capillary endothelial cells that constitute the physiological barrier between the general circulation and the brain.

Detailed Description of Related Art

The entry of drugs from the blood stream to the central nervous system (CNS), i.e., the brain and spinal cord, is restricted by the presence of high resistance tight junctions between brain capillary cells and by the apparently low rate of transport across these endothelial cells (Betz, A.L., et al., Ann. Rev. Physiol., 48:241 (1986); Pardridge, W.M., Ann. Rev. Pharmacol. Toxicol., 28:25 (1988)).

The tight junctions of the blood brain barrier (BBB) prevent diffusion of molecules and ions around the brain capillary endothelial cells. The only substances that can readily pass from the luminal core of the capillary to the abluminal tissues that surround the capillary are those molecules for which selective transport systems exist in the endothelial cells, as well as those compounds that are lipophilic (i.e., hydrophobic). In contrast, drugs, peptides and other

molecules that are neither lipophilic nor transported by specific carrier proteins are barred from entry into the brain, or their rates of entry are too low to be useful, thereby imposing a severe limitation upon the physician's ability to treat CNS disorders pharmacologically.

The carrier-mediated transcellular transport system mentioned above may have limited usefulness for therapeutic modalities under some circumstances. Transcytotic transport, in general, involves, first, the binding of molecules to specific carrier proteins on the surface of endothelial cells, and, second, the delivery of such molecules across the endothelial cells. Limitations on the usefulness of such a system for treatment of CNS disorders are based on the following considerations: (1) physiological carrier proteins may not function efficiently, or at all, with non-physiological drugs; (2) even where function occurs, the rate of transport of therapeutic agents will be limited by the rate of transport of the carrier; (3) the overall capacity of cerebral capillary endothelial cells to transport any therapeutic macromolecules may be simply too low to achieve therapeutic levels of certain drugs in the brain; and (4) once therapeutic macromolecules enter endothelial cells, depending on their nature, they might be delivered to any number of organelles, including lysosomes that contain a wide variety of hydrolytic enzymes. For these reasons, creating drug delivery systems that do not rely upon transcytosis will clearly be advantageous.

As tight junctions between brain capillary endothelial cells constitute a major part of the BBB, the possibility of modifying these junctions has been considered. It has been found that tight junctions,

including those of the BBB, can be disrupted by hyperosmotic solutions administered intra-arterially. For example, Polley et al., WO89/04663, published June 1, 1989, disclose the osmotic disruption of the interendothelial structure of the BBB by the intra-arterial administration of hypertonic solutions of mannitol, arabinose or glycerol as a means of introducing into the brain genetic material. Similarly, hyperosmotic solutions of urea have also been used to alter the BBB (Bowman, P.D. et al., Ped. Res., 16:335A (1982)).

Other chemical agents have been reported to disrupt endothelial or epithelial cell tight junctions when administered intravenously, including: 7-fluorouracil (MacDonell, L.A., et al., Cancer. Res., 38:2930 (1978)), degradation by membrane enzymes (Vincent, P.A., et al., Exp. Mol. Path., 48:403 (1988); Diener, H.M., et al., J. Immunol., 135:537 (1985)), aluminum salts (Zigler, Z.Y., et al., IRCS Med. Sci., 12:1095 (1984)), histamine (Meyrick, B., et al., Exp. Lung Res., 6:11 (1984)), thrombin (Siflinger-Birnboin, A., et al., Microvasc. Res., 36:216 (1988)), phorbol esters (Shiba, K., et al., Exp. Cell Res., 178:233 (1988)), and neutralization of the luminal anionic charge (Hart, M.M., J. Neuropathol. Exp. Neurol., 46:141 (1987)).

Although the above-listed modalities may disrupt tight junctions and thereby increase permeability of the BBB, problems attendant upon their use make them less than desireable. For example, intra-arterial perfusion with hyperosmotic solutions involves surgery, and this cannot be repeated on a regular basis. Further, concentrated sugar solutions may not be innocuous, and might be expected to have undesirable side effects. In addition, the aforementioned chemical

agents may not be useful for the treatment of chronic neurological disease, their effects on tight junctions are not always reversible, and, as they all are themselves powerful drugs, there is always the danger that their use will compromise the patient's health generally. For example, 7-fluorouracil is a powerful inhibitor of pyrimidine synthesis, and thus nucleic acid biosynthesis, in animals cells.

Thus, an important need still exists for means which transiently and reversibly disrupt tight junctions of the BBB in order that administered drugs can reach the brain from the general circulation, and which have no undesirable side effects of their own in the subject.

Attempts have been made to disrupt cell-cell adhesion by modifying the protein(s) responsible for such adhesion, collectively referred to as "cell adhesion molecules" (CAM). One class of CAM is termed "cadherin". "Cadherin" is the term applied to a family of glycoproteins found in most kinds of mammalian tissues and thought to be responsible for Ca^{2+}-dependent cell-cell adhesion, (Takeichi, M., Development, 102:639 (1988)). Three subclasses of cadherin have been identified, namely, E-cadherin (from epithelial tissues), P-cadherin (from placental tissues), and N-cadherin (from neural tissues) (Yoshida-Noro, C., et al. Dev. Biol., 101:19 (1984); Nose, A., et al., J. Cell Biol., 103:2649 (1986); Hatta, K., et al., Nature, 320:447 (1986)).

The different cadherins exhibit distinct tissue distribution patterns (Takeichi, U., (1988) above). E-cadherin, which was found to be distributed exclusively in epithelial cells of various tissues (Hatta, K., et al., Proc. Nat'l. Acad. Sci. (USA), 82:2789 (1985); Takeichi, 1988, above), appears to be

identical to uvomorulin (Hyafil, F., et al., Cell, 21:927 (1986)), chicken liver-cell adhesion molecule (L-CAM, Gallin, W.J., et al., Proc. Nat. Acad. Sci. (USA), 80:1038 (1983)), and cell-CAM 120/80 (Damsky, C.H., et al., Cell, 34:455 (1983)) in terms of biochemical properties (Cunningham, B.A., et al., Proc. Nat. Acad. Sci. (USA), 81:5787 (1984)) and tissue distributions (Thiery, J.-P., et al., Dev. Biol., 102:61 (1984)).

N-cadherin, which is expressed in various neural tissues including astrocytes (Hatta, K., et al., Devel. Biol., 120:215 (1987); Matsunega, M., et al., Nature, 334:62 (1988); Tomaselli, K.J., Neuron, 1:33 (1988)), shows 92% amino acid sequence homology between mammalian and avian homologs, shows from 40 to 50% similarity to epithelial E-cadherin and to placental P-cadherin of the same species, but was immunologically not cross-reactive with other cadherins within the same animal (Miyatani, S., Science, 245:631 (1989)).

Placental P-cadherin has also been cloned, and the deduced amino acid sequence of this glycoprotein was found to exhibit about 58% homology with epithelial E-cadherin (Nose, A., et al., EMBO J., 12:3655 (1987)).

Subsequent to the September 27, 1989 filing of the parent application, Heimark, et al. (Heimark, R.L., et al., J. Cell Biol., 110:1745 (1990) reported on the identification of a Ca^{2+}-dependent cell-cell adhesion molecule in aortic endothelial cells.

Although each of the aforelisted cadherins displays unique immunological and tissue distribution specifications, all have features in common: (1) a requirement for Ca^{2+} for cell adhesion function; (2) protection by Ca^{2+} from proteolytic cleavage; (3) similar numbers of amino acids, i.e., from about 723 to about 822; (4) similar masses, i.e., about 124 kdal.

for the glycoprotein; (5) substantial interspecies (50%-60%) overall sequence homology with interspecies homologies increasing to about 56% to 99% in the cytoplasmic region of the protein, suggesting that they constitute a gene family (Nose, A., 1987; Miysysni, D., et al., 1989); and (6) a common mechanism of action, namely, homophilic binding of cadherins on one cell to similar cadherins on the adjoining cell.

CAMs independent of Ca^{2+} are also known, for example, the 125K glycoprotein of Urushihara et al. (Urushihara, H., et al., Cell, 20:363 (1980)); N-CAM (Rutishauser, U., Nature, Lond., 310:549 (1984)); Ng-CAM (Grunet, M. et al., Proc. Nat'l. Acad. Sci. (USA), 81:7989 (1984)); L1 (Rathjien, F.G. et al., [redacted] J., 3:1 (1984)); G4 (Rathjien, F.G. et al., J. Cell Biol., 104:343 (1987)); and platelet glycoprotein PECAM-1 (CD 31) (Newman, P.J., Science, 247:1219 (1990)). Ca^{2+}-independent CAMs are known to exhibit certain properties of the Ca^{2+}-dependent CAMs. Thus, N-CAM and N-cadherin both promote retinal neurite outgrowth on astrocytes (Neugebauer, K.M., et al., J. Cell Biol., 107:1177 (1985)), and on Schwann cells (Bixby, J.L. et al., J. Cell Biol., 107:353 (1988)).

Monoclonal antibodies raised against epithelial E-type cadherins such as uvomorulin are known to disrupt the adhesion of several cell types, including embryo cells, cultured teratocarcinoma cells, hepatocytes, and MDCK kidney epithelial cells (Ogou, S.-I., et al., J. Cell Biol., 97:944 (1983); Yoshida-Noro, et al., (1984), above; Shirayoshi, Y., et al., Cell Struct. Funct., 11:285 (1986); Gallin, et al., (1983), above; Vestweber, D., et al., EMBO J., 4:3393 (1985); Johnson, M.H., et al., J. Embrol. Exp. Morphol., 93:239 (1986); Gumbiner, B., et al., J. Cell Biol., 102:457 (1986)).

However, prior to the present discoveries disclosed in the parent applications cadherins had not been found in brain capillary or other endothelial cells (see, Takeichi, et al. (1988), above). Further, the CAMs of microvascular endothelial cells had not yet been identified, nor had such molecules been localized specifically to brain capillary endothelial cells. Thus, until the present invention no means were known for transiently and reversibly disrupting tight junctions between microvascular endothelial cells, including those of the BBB, based upon an attack upon the CAM's of such cells that are responsible for tight junction formation and maintenance.

It has been hypothesized that the cadherins contain a common cell adhesion recognition (CAR) sequence. The CAR sequences of several cell and substratum adhesion molecules are known. Martin, G.R., et al., Ann. Rev. Cell Biol., 3:57 (1987) ; Ruoslahti, E., et al., Science, 238:491 (1987). In general, CAR sequences are composed of at least three amino acid residues. The most rigorously investigated CAR sequence is RGD which is found in laminin, fribronectin and other basement membrane components that are responsible for the binding of cells to the substratum.

Blaschuk, et al., in a paper to be published subsequent to the filing of the present application (Blaschuk, O., et al., J. Mol. Biol., in press, (1990)), disclose the presence of three potential cadherin CAR sequences in the first extracellular domains of liver CAM, E-, P-, and N-cadherin, namely, PPI, GAD and HAV. Blaschuk, et al. (Blaschuk, O., et al., Develop. Biol., 139:227 (1990)), also disclosed recently that synthetic peptides containing the HAV sequence inhibited two biological processes (compaction of 8-cell-stage mouse embryos and rate of neurite

outgrowth on astrocytes) that are known to be mediated by cadherins. Effective peptides in these assays were LRAHAVDVNG and AHAVSE; PPI-containing peptides were without effect. However, Blaschuk et al. provide no guidance for determining the regions flanking the HAV tripeptide that are critical for cell-cell adhesion. In the BBB disrupting peptides of the present invention detailed below, we have observed that the mere presence of the HAV sequence in a small cadherin-derived peptide is not the sine qua non for a composition effective to prevent cell-cell adhesion. Indeed, it should be emphasized that neither Blaschuk et al. nor any other publication known to the present inventors suggest that cadherin sequences containing HAV or SHAVS sequences would be effective in opening tight junctions and piercing blood brain barriers formed by E-cadherins in brain microvascular endothelial cells.

SUMMARY OF THE INVENTION

It has now been discovered that molecules homologous to, and immunologically related to, cadherin cell adhesion molecules are present on brain and non-brain microvascular endothelial cells, such that

junctions between such endothelial cells can be reversibly opened so as to permit passage of therapeutic drugs by the use of polypeptide and antibody compositions that compete with such cell adhesion molecules for binding to such cells.

It is therefore an object of this invention to provide the identity of microvascular endothelial cell adhesion molecules.

Another object of this invention is to provide DNA sequences of genes, and plasmids containing same, coding for the expression of all or a cell-binding portion of microvascular endothelial cell adhesion molecules.

Yet another object of this invention is to provide means to identify those sequences of cell adhesion molecules responsible for the tight binding of adjoining endothelial cells.

A further object is to provide therapeutic compositions comprising polypeptides derived from cell adhesion molecules that reversibly disrupt cell-cell adhesion.

Still another object of this invention is to provide therapeutic compositions comprising polyclonal or monoclonal antibodies or fragments thereof directed against endothelial cell adhesion molecules, or against polypeptides representing cell binding regions thereof, that reversibly disrupt endothelial cell-cell adhesion.

Yet another object of this invention is to provide therapeutic formulations comprising therapeutic drugs conjugated with blood-brain barrier-disrupting compositions of this invention, that are capable of entering the central nervous system following disruption of the blood-brain barrier.

These and other objects of this invention will become clear by reference to the following description

of the invention and to the appended claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to chicken N-cadherin.

Figure 2 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to mouse P-cadherin.

Figure 3 illustrates the cDNA sequence for the MDCK cell adhesion molecule homologous to mouse E-cadherin.

Figure 4 illustrates the restriction sites in the bovine endothelial cell N- (4-1 to 4-5) and P-cadherin (4-6 to 4-8) cDNA sequences and in the MDCK E-cadherin (4-9 to 4-14) cDNA sequence.

Figure 5 shows the staining of a mouse brain thin section by an antibody raised against a fusion protein derived from amino acids 9-96 of MDCK E-cadherin containing an HAV region.

Figure 6 is a repeat of the experiment of Fig. 5, except that the antibody was raised against the entire E-cadherin molecule.

Figure 7 illustrates the effects of an 18-mer HAV-containing polypeptide on the resistance of tight junction monolayers of MDCK epithelial cells.

Figure 8 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight junction monolayers MDCK epithelial cells.

Figure 9 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight-junction monolayers of brain microvascular endothelial cells.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that cell adhesion molecules with characteristics of cadherins are present on the surfaces of brain capillary endothelial cells and of microvascular endothelial cells of non-brain origins. The present invention is based on the discovery that a polypeptide composition comprising cell binding domains of endothelial cell adhesion molecules may compete against such molecules for binding to such cells, such that by this means the junctions between such cells could be reversibly opened, thereby permitting penetration by therapeutic agents. The present invention also discloses that polyclonal or monoclonal antibodies (or fragments thereof) raised against endothelial cell adhesion molecules or cell-binding domains thereof may also compete for endothelial cell surface binding sites, and, by this means, reversibly disrupt junctions between endothelial cells, thereby permitting entry into the central nervous system of therapeutic agents.

In order to obtain compositions useful for disrupting tight junctions between microvascular endothelial cells, the cell adhesion molecules responsible for such junctions were identified.

The endothelial cell cadherins disclosed herein exhibit one or more of several characteristics of E-, P- and N- cadherins, including: characteristics of a transmembrane integral protein, with cytoplasmic, hydrophobic plasma membrane, and extracellular regions; intraspecies DNA sequence homologies of greater than about 50% for the entire molecule; immunological cross-reactivity with antibodies raised against non-endothelial cell cadherins; and containing cell-binding domains. "Immunologically related to" means that these cadherin-like molecules cross-react with antibodies

raised against non-endothelial cell cadherins.

E-cadherin-like molecules were localized in brain by immunofluorescence. Cryostat sections of mouse brain were labeled with a rabbit antibody prepared against E-cadherin, and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin. There is clear labeling of a capillary in brain sections as shown by immunofluorescence microscopy. Endothelial cells in liver and kidney were not stained by this procedure.

cDNAs coding for the expression of bovine microvascular endothelial cell (BMEC) cadherins were cloned and sequenced as described below, and the partial sequence of N-cadherin and P-cadherin are disclosed herein in Figures 1 and 2, respectively. In addition, as MDCK dog kidney epithelial cells are known to employ E-cadherin to form high resistance tight junctions, and as the present invention discloses that brain capillary endothelial cell adhesion molecules include E-type cadherin, the DNA of this cadherin was also cloned; its complete DNA sequence is disclosed herein (Fig. 3).

N-, P- and E-cadherin-type clones described herein were deposited in the American Type Culture Collection on September 26, 1989, and were assigned the following accession numbers:

Clone Designation	Accession No.
N-cadherin-type clones	
pUC19-bNCad 10A	40667
pUC19-bNCad 39A	40669
P-cadherin-type clones	
pUC18-bPCad 3B-10	40668
pUC19-bPCad 9B	40670
E-cadherin-type clones	
pBluescript MDCKECad 45-30E	40671

The cloning of cadherins was accomplished by taking advantage of the fact that the cadherins characterized thus far are transmembrane glycoproteins, the cytoplasmic domains of which are highly conserved, that is, are highly homologous.

Two degenerate oligonucleotides flanking the 42-amino acid coding region in the cytoplasmic domain were selected to serve as primers for polymerase chain reaction (PCR) using either BMEC cDNA or MDCK cDNA as templates. The PCR reactions were carried out essentially according to Saiki, R. K. et al., Science, 239:487 (1988), which is incorporated herein by reference.

The cloned PCR products from each cell type were sequenced essentially according to the method of Sanger, F. et al., Proc. Nat'l. Acad. Sci. (USA), 74:5463 (1977), which is incorporated herein by reference.

It was discovered that BMEC cadherins are of two types - one homologous to chicken N-cadherin (neuronal type, see, e.g., Hatta, K., et al., J. Cell Biol., 106:873 (1988)) and the other homologous to mouse P-cadherin (placental type, see e.g., Nose, A., et al., (1987) above). It has also been found that there are two species of cadherins in MDCK cells - one homologous

to mouse E-cadherin (see, e.g., Nagafuchi, A., et al., Nature, 329:341 (1987)) and the other homologous to mouse P-cadherin (Nose, et al. (1987), above).

The PCR products were then used as probes to isolate the BMEC and MDCK cadherin cDNA clones as follows. A cDNA library was constructed essentially according to Gubler et al. (Gubler, U. et al., Gene, 25:263 (1983), which is incorporated herein by reference), using poly $(A)^+$RNA isolated from either BMEC or MDCK cells. The cDNA was ligated via EcoRI adaptors into gt10 arms (BMEC) or ZAP^R (from Stratagene, Inc., La Jolla, CA) vector arms (MDCK). cDNA libraries containing $5 \times 10^5 - 1.5 \times 10^6$ independent cDNA clones were screened using radiolabeled PCR products (Benton, W.D. et al., Science, 196:180 (1987), which is incorporated herein by reference). Northern blot analysis (Maniatis, T. et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982) may be used to determine whether each cDNA species cloned hybridizes to a single mRNA species, as well as the tissue distributions of each cDNA species.

cDNA clones for each cadherin were sequenced by the method of Sanger et al. (1977) above.

The partial restriction maps for each cDNA clone based on their sequences are shown in Fig. 4. Some of these restriction sites were confirmed by restriction enzyme digestions, including Hind III, Pst I, Kpn I, Bgl II for N-cadherin; Pvu II, Sac I and Pst I for P-cadherin; Pst I, Pvu II, BamH I, and Sac I for E-cadherin.

In order to test whether the cloned E-cadherin cDNA contains all the information necessary for cadherin function, full-length E-cadherin cDNA joined to a suitable promoter may be introduced into mouse

L-cells that have very little endogenous cadherin activity (Nagafuchi, et al. (1987), supra). To test for expression of E-cadherin in transfectants derived from the introduced cDNA, transfected L-cells may be tested for Ca^{2+}-dependent aggregating activity. The extent of this aggregating activity should be closely correlated with the amount of E-cadherin expressed (Takeichi, M. (1988), supra). This same technique may be used for testing cDNAs encoding bovine endothelial N- and P-cadherins, according to the method of Hatta, et al. (Hatta, K., et al. (1988), supra).

In order to identify cell binding domains in, for example, MDCK E-type cadherin, L-cells may be first transfected as above with a cDNA of a size sufficient to cause Ca^{2+}-mediated aggregation of transfectants. A series of deletion mutants comprising truncated cDNA species missing different regions of the extracellular domain may be prepared by restriction enzyme digestion and proper end filling or exonuclease digestion to make the deletions in the proper coding frames. These deletion mutants can then be tested for their ability to express in L-cells a protein causing Ca^{2+}-dependent aggregation. By correlating a loss of aggregation with deletion of particular fragments, the regions important for cell binding may be determined. A variety of polypeptides corresponding to binding regions of cadherins, as deduced from the nucleotide sequences of deleted cDNA, may be synthesized chemically using an automated peptide synthesizer such as that of Applied Biosystems, Inc., Foster City, CA, or expressed by recombinant DNA methods. Effective polypeptides may be of varying lengths, depending upon the natures of junctions being disrupted and the cell adhesion molecule present.

Nucleotide, and corresponding amino acid, sequences of cadherins may be analyzed to detect homologous regions. Applying this technique to bovine endothelial cell N- and P-cadherins and to epithelial cell E-cadherin, we have determined that, in the amino acid 80 region of each of these cadherins, there is conserved a triplet HAV (His-Ala-Val) region. We have deduced that this HAV region may be a common cell adhesions recognition (CAR) sequence.

We have chemically synthesized the following polypeptides, each of which containing the HAV sequence:

6-mer(78-83)	NH_2-SHAVSS-$CONH_2$
11-mer(76-86)	NH_2-LYSHAVSSNGN-$CONH_2$
17-mer(74-90)	NH_2-YILYSHAVSSNGNAVED-$CONH_2$
18 mer(69-86)	NH_2-EQIAKYILYSHAVSSNGN-$CONH_2$
20-mer(71-90)	NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$

and have tested each for efficacy in opening brain endothelial cell tight junctions in the BBB model disclosed in copending United States application Serial No. 07/413,274, and also on kidney epithelial cell tight jucntions..

Polyclonal antibodies raised in rabbits and monoclonal antibodies derived from hybridomas may be generated against each of the chemically-synthesized polypeptides by standard methods. (Harlow, E., et al., "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988; Goding, J.W., "Monoclonal Antibodies: Principles and Practice", Academic Press, N.Y. 1986). In addition, recombinant antibodies may be prepared. Fragments of antibodies, e.g., Fc, Fab, F(ab)', may be prepared by standard methods.

We have cloned and sequenced fusion proteins derived from amino acids 9-96 of MDCK E-cadherin

containing the HAV region. A polyclonal antibody prepared against this fusion protein stained rat (Fig.55) mouse brain sections as well as did an antibody raised against the entire E-cadherin (Fig. 6). A polyclonal antibody raised against a fusion protein derived from amino acids 9-37 failed to stain brain sections. These results indicate that the key cell-binding domain of E-cadherin lies in the region of amino acids 37-96.

The ability of CAM-derived polypeptides containing cell-binding domains, and the corresponding polyclonal and monoclonal antibodies, of the invention to disrupt tight junctions may be tested in _in vitro_ and _in vivo_ models of high resistance tight junctions and in animal models. Monolayers of MDCK dog kidney epithelial cells, that are known to contain high resistance tight junctions (Gumbiner, B., _J. Cell Biol._, 102:457 (1986)), can be used to test for the ability of the polypeptides and corresponding antibodies of the present invention to disrupt such tight junctions.

Polyclonal antibodies prepared as described above may also be used in conjunction with Western blotting (Old, R.W., _et al._, _Principles of Gene Manipulation_, 3d ed., Blackwell, Oxford, 1985, p. 10) and a variety of tissue extracts in order to identify cell adhesion glycoproteins in such extracts.

Another embodiment of the present invention is in drug delivery systems. Conjugates between therapeutic drugs and agents that affect cell adhesion molecule function in brain capillary endothelial cells may be used to deliver therapeutic drugs to the CNS. For example, a polypeptide derived from a cell adhesion molecule that contains within its amino acid sequence a cell-binding domain, or antibodies thereto, may be conjugated in biologically-active form to a therapeutic

modality. Such conjugates may have the dual effect of opening the BBB and delivering the therapeutic agent to the brain side of the BBB. Delivery of therapeutic drugs to the CNS, either alone or conjugated to agents that disrupt cell-cell adhesion, may be accomplished by administering such drugs to a subject either simultaneously with or subsequent to the administration of the agents of this invention that disrupt the tight junctions of the BBB. Examples of therapeutic modalities that may be delivered to the brain by the cell adhesion disruption compositions of this invention include Nerve Growth Factor, anti-Parkinsonian drugs, and brain enzymes known to be missing in sphingolipidoses, e.g., Tay-Sachs disease. Means of chemically conjugating protein or polypeptide carriers to therapeutic agents such that the biological integrity of the therapeutic agent is not compromised and such that the therapeutic agent is readily cleaved from the carrier by enzymes present on or within endothelial cells (e.g., amidases, esterases, disulfide-cleaving enzymes), are well known in the art. It is also apparent that these therapeutic conjugates may be delivered to endothelial cells in encapsulated form (e.g., in liposomes) or as microsuspensions stabilized by pharmacological excipients.

It is known (Jain, R.K., J. Natn'l Cancer Inst., 81:570 (1989)) that many solid tumors develop internal barriers, including high pressure zones and collapsed blood vessels, that make it difficult for blood-borne chemotherapeutic agents to reach the tumor's inner core. The barrier problem is particularly troublesome with therapeutic products drawn from the human immune system, such as monoclonal antibodies conjugated with chemotherapeutic agents, interleukin-2, interferon and activated killer T-lymphocytes, because of their large

size. Thus, in another embodiment of this invention, compositions that disrupt the junctions between endothelial cells, particularly the relatively small peptides that contain one or more cell-binding regions of cell adhesion macromolecules, may be used to enhance drug delivery to tumors with depressed blood flow.

It has been theorized that cancer cells metastasize by secreting soluble cadherins variously to open tight junctions in cells that block their movement and to prevent their being bound to such cells. We consider it likely that antibodies raised against these cadherins, which are derived from extracellular domains of the cadherins disclosed in this invention, may provide a therapeutic modality that inhibits or prevents cancer cell metastases.

In another embodiment, the compositions of this invention may also be used to provide penetration for chemotherapeutic agents of other well-known blood-tissue barriers, such as blood-testis barriers and blood-retina barriers. The latter barrier is known to prevent the efficient transport of, for example, administered antibiotics to the retina from the general circulation. The cell adhesion disrupting compositions of this invention may, thus, be used in conjunction with the administration of antibiotics to treat retinal infections.

The following examples are illustrative of several embodiments of this invention, and should not be construed in any way as limiting the invention as recited in the claims.

EXAMPLE 1

EFFECTS OF HAV-CONTAINING POLYPEPTIDES ON TIGHT JUNCTIONS OF MDCK EPITHELIAL AND BOVINE ENDOTHELIAL CELLS

The BBB model of copending U.S. Serial No. 07/413,332 was used to examine the effects of polypeptides containing the HAV region on the tight junctions of monolayers of MDCK epithelial cells and bovine capillary endothelial cells as determined by resistance measurements across the monolayers.

The polypeptide was added to the cells either from the apical side (top) or basolateral side (bottom), as shown in the following sketch.

APICAL

EPITHELIAL CELLS	ENDOTHELIAL CELLS
Gut Side	Blood Side
Blood Side	Brain Side

BASOLATERAL

Figure 7 illustrates the effects of various concentrations of the aforementioned 18-mer polypeptide on resistance of MDCK epithelial cells. At the lowest concentration tested, 0.5 mg/ml, resistance was markedly decreased. The polypeptide was more effective when added from the basolateral side, but at high concentrations was quite effective even when added from the apical side. These data indicate that the 18-mer is effective in making tight junctions permeable. The 20-mer was similarly effective, and a 17-mer less effective.

Figure 8 illustrates the effects of the aforementioned 11-mer and 18-mer on MDCK cell resistance when added from either the apical or basolateral side of the monolayers. The concentration of polypeptide was about 1 mg/ml. The 11-mer (as well

as the 6-mer data not shown) was virtually without effect. With the 18-mer, resistance was almost totally abolished by about 6 hours, indicating disruption of tight junctions. That the effect of the 18-mer is reversible is indicated by the "wash-out" experiment. When the 18-mer was washed out of the MDCK cells at 6 hours, resistance recovered to a substantial extent over the next 21 hours. This recovery was particularly pronounced when the 18-mer had originally been added from the basolateral side of the monolayers. The 20-mer produced results similar to those of the 18-mer, and the 17-mer was effective, but somewhat less so.

Figure 9 illustrates the effect of 1 mg/ml of the 11-mer and 18-mer on high resistance monolayer cultures of brain endothelial cells (see copending United States Serial No. 07/413,332 for method of preparation). As with MDCK cells, the 11-mer (and the 6-mer) failed to reduce resistance values over a 48-hour period of observation. In contrast, the 18-mer (as well as the 20-mer) decreased resistance values markedly when added from either the basolateral or apical side, but the effect of the polypeptide was more rapid and more pronounced when it was added from the basolateral side; the 17-mer was less effective.

The conclusion of these experiments is that a particular set of peptides (but not all peptides) centered around the HAV region of E-cadherin are effective in opening tight junctions of brain endothelial cell blood-brain barriers, and also of epithelial cells that form such junctions ("gut barrier"). Both the length and compositon of the amino acid region flanking the HAV triplet thus appear to play a role in the efficacy of such compositions.

While the aforementioned embodiments represent the preferred embodiments of the invention, those skilled

in the art may, without undue experimentation, devise other executions of the compositions and methods of use of this invention without departing from the concept and spirit inherent therein.

What is claimed is:

1. A composition for opening tight junctions between microvascular endothelial cells of a subject, whereby means are provided for a drug to cross the permeability barrier imposed by such junctions, comprising an agent capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted.

2. A composition of claim 1, wherein said cell adhesion molecule exhibits at least about 50% sequence homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

3. A composition of claim 1, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

4. A composition of claim 1, wherein the microvascular endothelial cells are brain capillary endothelial cells.

5. A composition of claim 2, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

6. A composition of claim 3, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

7. A composition of claim 5, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

8. A composition of claim 7, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

9. A composition of claim 8, wherein said cell-binding domain contains an HAV amino acid sequence.

10. A composition of claim 9, wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

11. A composition of claim 9, wherein said amino acid sequence is

NH2-EQIAKYILYSHAVSSNGN-$COHN_2$.

12. A composition of claim 9, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.

13. A composition of claim 9, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

14. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

15. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

16. A composition of claim 15, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

17. A composition of claim 16, wherein said cell-binding domain contains an HAV amino acid sequence.

18. A composition of claim 17, wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

19. A composition of claim 17, wherein said amino acid sequence is

NH2-EQIAKYILYSHAVSSNGN-$COHN_2$.

20. A composition of claim 17, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.

21. A composition of claim 17, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

22. A composition of claim 5 or 6 in a pharmaceutically-acceptable vehicle.

23. A method for opening tight junctions between microvascular endothelial cells of a subject, comprising the step of administering to the subject an agent, in an effective amount and in a pharmaceutically-acceptable vehicle, capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted and whereby means are provided for a drug to cross permeability barriers imposed by such tight junctions.

24. A method of claim 23, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

25. A method of claim 23, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

26. A method of claim 23, wherein the microvascular endothelial cells are brain capillary endothelial cells.

27. A method of anyone of claims 23-25, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

28. A method of claim 27, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

29. A method of claim 28, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

30. A method of claim 29, wherein said cell-binding domain contains an HAV amino acid sequence.

31. A method of claim 30 wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

32. A method of claim 30, wherein said amino acid sequence is

NH2-EQIAKYILYSHAVSSNGN-$COHN_2$.

33. A method of claim 30, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.

34. A method of claim 30, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

35. A method of claim 27, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

36. A method of claim 28, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said fragment of said cell adhesion molecule.

37. A method of claim 36, wherein said cell adhesion fragment includes within its amino acid sequence a cell-binding domain.

38. A method of claim 37 wherein said cell-binding domain contains an HAV amino acid sequence.

39. A method of claim 38, wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

40. A method of claim 38, wherein said amino acid sequence is

NH2-EQIAKYILYSHAVSSNGN-$COHN_2$.

41. A method of claim 38, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.

42. A method of claim 38, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

43. A drug delivery composition comprising a conjugate between a therapeutic drug and an agent capable of reacting with at least one type of a cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is

disrupted by said agent, whereby means are provided for said drug to cross permeability barriers imposed by such tight junctions, in a pharmaceutically-acceptable vehicle.

44. A drug delivery composition of claim 43, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

45. A drug delivery composition of claim 43, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

46. A drug delivery composition of claim 43, wherein the microvascular endothelial cells are brain capillary endothelial cells.

47. A drug delivery composition of any one of claims 43-45, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

48. A drug delivery composition of claim 47, wherein said agent comprises a fragment of said cell adhesion molecule.

49. A drug delivery composition of claim 48, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

50. A drug delivery composition of claim 49, wherein said cell-binding domain contains an HAV amino acid sequence.

51. A drug delivery composition of claim 50, wherein said amino acid sequence is

$$NH_2\text{-YILYSHAVSSNGNAVED-}CONH_2 .$$

52. A drug delivery composition of claim 50, wherein said amino acid sequence is

$NH2$-EQIAKYILYSHAVSSNGN-$COHN_2$.

53. A drug delivery composition of claim 50, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.

54. A drug delivery composition of claim 50, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

55. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

56. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

57. A drug delivery composition of claim 56, wherein said cell adhesion molecule fragment contains within its amino acid sequence a cell-binding domain.

58. A drug delivery composition of claim 56, wherein said cell-binding domain encompasses an HAV amino acid sequence.

59. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

60. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH2-EQIAKYILYSHAVSSNGN-$COHN_2$.

61. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.

62. A drug delivery composition of claim 58, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

63. A drug delivery composition of claim 43, wherein said conjugate comprises a physiologically-cleavable covalent bond.

64. A drug delivery composition of claim 43, wherein said conjugate is encapsulated within a physiologically-compatible particle.

65. A drug delivery composition of claim 64, wherein said particle comprises a liposome.

FIG. 1a.

Partial cDNA sequence for the bovine endothelial N-cadherin

GAATTCGAAC	CCCTTCGTTT	CATTATGCAA	GACTGGATTT	CCTGAAGATG	TGTACAGTGC	60
AGTCTTGTCC	CGGGATGTGC	TGGAAGGACA	GCCCCTTCTC	AATGTGAAGT	TTAGCAACTG	120
CAATGGGAAA	AGAAAAGTAC	AGTATGAGAG	CAGCGAGCCA	GCAGATTTTA	AGGTGGATGA	180
AGATGGCATG	GTGTATGCCG	TGAGAAGCTT	CCCCCTCTCA	TCTGAACACT	CGAAGTTCCT	240
GATATACGCT	CAAGACAAAG	AGACTCAGGA	AAAGTGGCAA	GTAGCAGTAA	AACTGAGCCT	300
CAAACCAGCC	CTACCTGAGG	ATTCAGTGAA	GGAATCACGA	GAAATAGAAG	AAATAGTGTT	360
TCCAAGACAA	GTGACTAAGC	ACAATGGCTA	CCTGCAGAGG	CAGAAGAGAG	ACTGGGTTAT	420
CCCTCCCATC	AACTTGCCAG	AAAACTCCAG	AGGGCCTTTT	CCTCAAGAGC	TCGTCAGGAT	480
CAGATCCGAT	AGAGATAAAA	ACCTTTCTCT	GCGGTACAGC	GTAACTGGGC	CAGGAGCTGA	540
CCAGCCTCCA	ACTGGTATCT	TCATTATCAA	CCCCATCTCA	GGTCAGCTGT	CAGTAACCAA	600
GCCTCTGGAT	CGTGAGCTGA	TAGCCCGGTT	TCATTTGAGG	GCACATGCAG	TGGATATTAA	660
TGGAAACCAA	GTGGAGAACC	CCATCGACAT	TGTCATCAAC	GTTATTGACA	TGAATGATAA	720
CAGACCTGAG	TTCTTACACC	AGGTTTGGAA	TGGGACAGTT	CCTGAGGGAT	CAAAGCCGGG	780
AACATATGTG	ATGACGGTCA	CTGCGATTGA	TGCTGACGAT	CCAAATGCCC	TCAATGGGAT	840

FIG. 1b.

```
GTTGAGGTAC AGAATCCTGT CCCAGGCGCC AAGCACCCCT TCGCCCAACA TGTTTACAAT  900
CAACAATGAG ACTGGGGACA TTATCACGGT GGCAGCTGGA CTTGACAGAG AAAAAGTACA  960
ACAGTATACG TTAATAATTC AAGCTACAGA CATGGAAGGC AATCCCACAT ATGGCCTTTC 1020
CAACACAGCC ACGGCTGTCA TCACGGTGAC AGATGTCAAC GACAATCCTC CGGAGTTTAC 1080
TGCCATGACG TTCTATGGTG AAGTCCCTGA AAACAGGGTA GATGTCATCG TCGCTAATCT 1140
AACAGTGACA GATAAGGATC AGCCCCACAC ACCGGCCTGG AACGCCATCT ACAGAATCAG 1200
CGGTGGAGAC CCCGCCGGCC GCTTTGCCAT TCAAACTGAC CCCAACAGCA ACGACGGTTT 1260
AGTCACCGTA GTAAAACCAA TCGACTTTGA AACAAATAGG ATGTATGTCC TTACTGTCGC 1320
TGCAGAAAAT CAAGTGCCAT TAGCCAAGGG TATTCAGCAT CCACCTCAGT CAACTGCGAC 1380
TGTGTCTGTC ACAGTTATCG ATGTGAATGA AAATCCTTAT TTTGCCCCAA ATCCAAAGAT 1440
CATTCGCCAA GAAGAAGGCC TTCACGCCGG TACCGTGTTA ACAACGTTTA CTGCTCAGGA 1500
CCCAGATCGA TATATGCAGC AAAATATCAG ATACACCAAA TTATCCGATC CTGCAAACTG 1560
GCTAAAAATA GACTCTGTGA ATGGGCAGAT AACTACCATT GCTGTTTTGG ACAGAGAATC 1620
ACCGAATGTG AAAGCCAATA TATACAATGC TACTTTCCTT GCTTCTGACA ATGGAATCCC 1680
TCCTATGAGT GGAACGGGAA CACTGCAGAT CTATTTACTT GATATTAATG ACAATGCCCC 1740
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FIG. 1c.

```
TCAAGTGTTA CCTCAAGAGG CAGAGATTTG TGAAACTCCG GACCCCAATT CAATTAACAT 1800
CACAGCACTT GATTATGACA TTGATCCAAA TGCTGGACCA TTTGCTTTTG ATCTTCCTTT 1860
GTCTCCAGTG ACTATTAAGA GAAATTGGAC CATCACTCGG CTTAATGGTG ATTTTGCTCA 1920
GCTTAACTTA AAGATAAAAT TTCTTGAGGC CGGGATCTAC GAAGTTCCAA TCATAATCAC 1980
AGATTCGGGT AATCCTCCCA AATCGAATAT CTCCATCCTT CGGGTGAAGG TTTGCCAGTG 2040
TGATTCCAAC GGGGACTGCA CAGATGTGGA TCGAATTGTG GGAGCAGGGC TGGGCACCGG 2100
CGCCATCATC GCCATCCTGC TTTGCATCAT CATCCTGCTC ATTCTCGTTC TGATGTTCGT 2160
GGTATGGATG AAACGCCGGG ATAAAGAACG CCAGGCCAAA CAACTTTTAA TTGATCCAGA 2220
AGATGATGTA AGAGATAATA TTTTAAAATA TGATGAAGAA GGTGGAGGAG AAGAAGACCA 2280
GGACTACGAT TTGAGCCAGC TCCAGCAGCC TGATACGGTA GAGCCAGATG CCATCAAGCC 2340
AGTTGGAATC CGACGGTTGG ATGAGAGGCC CATCCATGCG GAGCCCCAGT ACCCGGTTCG 2400
ATCTGCAGCC CCACACCCAG GGGACATCGG GGACTTCATT AATGAGGGCC TTAAAGCTGC 2460
TGACAACGAT CCCACCGCTC CGCCCTACGA CTCCCTCTTA GTCTTTGACT ATGAAGGCAG 2520
TGGCTCCACG GCCGGGTCCT TGAGCTCCCT TAATTCCTCC AGTAGTGGAG GTGAGCAGGA 2580
CTATGACTAT CTGAACGACT GGGGGCCCCG CTTCAAGAAA CTCGCTGACA TGTACGGTGG 2640
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FIG.1d.

```
AGGTGATGAC TGAACTTCAG GGTGAACTTG GTTTTTGGAC AAGTACAAAC AATTGCAACT 2700
GATATTCCCA AAAAGCATTC AGAAGCTAGG CTTTAACTTT GTAGTCTACT AGCACAGTGC 2760
TTGCTGGAGG CTTTGGCAGA GGCTGCAAAC CAATTTGGGC TCAGAGGGAA TATCGGTGAT 2820
CCAATACTGT TTGGAAAACA CTGAGCTCAG TTACACTTGA ATTTTACAGT ACAGAAGCAC 2880
TGGGATTTTA TGTGCCTTTT TGTACCTTTT TCAGATTGGA ATTAGTTTTA TGTTTAAGGC 2940
TTTAATGGTA CTGATTTCTG AAATGATAAG TAAAAGACAA AATATTTTGT GGTGGGAGCA 3000
GTAAGTTAAA CCATGATATG CTTCGACACG CTTTTGTTAC ATCGCATTTG CTTTTATTAA 3060
AAATATGGAA TTAAACAGAC AAACCAACCA CTCATGGAGC AATTTTATTA CCTTGGGGGC 3120
TGAGACCATG AGATTGGAAA ATGTACATTA TTTCTAGTTT TAGACTTTAG TTTCTTGTTT 3180
TGTTTTTTTT TTCCACTAAA ATCTTAAAAC TTACGCAGCT GGTTGCAAAT AAAGGGAGTT 3240
TTCATATCAC CAATTTGTAG CAAAATTGAA TTTTTTCATA AACTAGAATG TTAGACACAT 3300
TTTGGTCTTA ATCCATGTAC ACTTTTTTAT TTACTGTATT TTTTCCACTT CACTGTAAAA 3360
ATGGTATGTG TACATAATGT TTTATTGGCA TAGTCTATGG AGAAGTGCAG AAACTTCAGA 3420
ACATGTGTAT GTATTATTTG GACTATGGAT TCAGGTTTTT TGCATGTTTA TATCTTTCGT 3480
TATGGATAAA GTATTTACAA AACAAAGTGA CATTTGATTC AATTGTTGAG CTGTAGTTAG 3540
AATACTCAAT TTTTAATTTT TTAATTTTTT TTATTTTTTA TTTTCTCTTT TTGTTTGGGG 3600
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AGGGAGAAAA GTTCTTAGCA CAAATGTTTT ACATAATTTG TACCAAAAAA AAACAAAAAA  3660
AAAGGAAAGA CAAGAAATGA AAGGGGTGAC CTGACACTGG TGGTACTACT GCAGTGTGTG  3720
TTTTTAAAAA AAAATGAAAA AAAAAAAGCT TTTAAACTGG AGAGACTTCT GACAACAGCT  3780
TTGCCTCTGT ATTGTGTACC AGAATATAAA TGATACACCT CTGACCCCAG CGTTCTGAAT  3840
AAAATGCTAA TTTTGGAAAA AAAAAAAAAA AAAAA                             3875
```

FIG. 1e.

FIG. 2a.

partial cDNA sequence for the bovine endothelial P-cadherin

```
GAATTCGAAC CCCTTCGCTG AGAACACAGT GAGCCACGAG GTGCAGAGGC TGACAGTGAC    60
TGATCTGGAC GCCCCTAACT CACCAGCATG GCGTGCCACC TACCGCATCG TGGGAGGTGA   120
CAACGGGGAC CATTTTACCA TCACTACTGA CCCCGAGAGC AACCAGGGTA TCCTGACCAC   180
CCAGAAGGGC TTGGATTTTG AGGCCAAAAC CCAGCACACC CTGTACGTCG AAGTGATCAA   240
CGAGGTTCCC TTTGTGGTGA AACTCCCGAC CTCCACAGCC ACCGTAGTGG TCCTCGTGGA   300
GGATGTGAAT GAGCCACCCG TGTTTGTCCC CCCGTCCAAA GTCATCGAAA TCCAGGAGGG   360
CATCTCCACT GGGGAGCCTA TTTGTGCCTA CACTGCACGG GACCCAGACA AGGGGAGTCA   420
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FIG.2b.

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GAAGATCAGT TACCACATCC TGAGAGACCC AGCAGGGTGG CTAGCGATGG ACCCAGACAG   480
TGGACAAGTC ACTGCCGCAG GGGTCTTGGA CCGTGAGGAT GAGCAGTTTG TGAGAAACAA   540
CATCTACGAA GTCATGGTCT TGGCCACAGA TGATGGGAGC CCTCCCACCA CTGGCACAGG   600
GACCCTCCTG CTAACACTGA TGGACATCAA TGACCACGGT CCGGTCCCCG AGCCCCGTCA   660
GATCACCATC TGCAACCAAA GCCCTGTGCC CCAGGTGCTA AACATCACAG ACAAGGACTT   720
GTCCCCCCAC ACTGCCCCTT TCCAGGCCCA ACTCACACAT GACTCGGACG TCTATTGGAC   780
AGCAGAAGTC AACGAGAAAG GAGACGCAGT AGCCTTGTCC CTGAAGAAGT TCCTAAAGCA   840
AGGCGAATAC GATGTGCACC TTTCCCTGTC CGACCACGGC AACAAGGAAC AGCTGACAGT   900
GATCAGAGCC ACCGTGTGTG ACTGCCACGG CAACATGGTG ACCTGCCGGG ACCCCTGGAC   960
GTGGGGTTTC CTCCTCCCCA TCCTGGGTGC TGCCCTGGCT CTGCTGCTCC TTCTGCTGGT  1020
GCTCCTATTC TTGGTGAGAA AGAAACGGAA GATCAAGGAA CCCCTTCTCC TCCCAGAAGA  1080
TGATACCCGT GACAACGTCT TCTACTACGG CGAAGAGGGG GGTGGCGAGG AGGACCAGGA  1140
CTATGACATC ACCCAGCTCC ACCGGGGTCT GGAGGCCCGG CCTGAGGTGG TTCTCCGCAA  1200
CGATGTGGCA CCATCCTTCA TCCCCACACC CATGTACCGT CCTCGGCCAG CCAACCCAGA  1260
TGAAATCGGC AACTTCATCA TTGAGAACCT GAAGGCAGCC AACACAGACC CCACGGCCCC  1320
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GCCCTACGAC TCCCTGTTGG TGTTCGACTA TGAGGGCAGT GGCTCCGATG CCGCCTCTCT  1380
GAGCTCGCTC ACCTCCTCAA CCTCTGACCA GGACCAAGAC TACAACTATC TGAATGAGTG  1440
GGGCAGCCGC TTCAAGAAGC TGGCGGACAT GTACGGCGGG GGCCAGGACG ACTAGGACTC  1500
CCTAAACGCC GGGCTGCAGC AGCGTCTCCA AGGGGTCACT ATCCCCACGT TGGCCAAGGA  1560
CTTTGCAGCT TGTTGAGAAT TGGCCTTAGC AACTTGGAGG GAAGAGGCCT CGAAACTGAC  1620
CTCAAAGGGG CAGGTCTCTA TGCCTTTCAG AACGGAGGAA CGTGGGCAGT TTGATTTCAA  1680
CAGTGAGCAC CTCTTAGCCT AAGCCAGGGC TGCTCAATTT CTGGGAGTCT CCTCGCTACC  1740
ATAAAATGCT CAGCGCTGGG TCCTGGGTTT TGACTGACTC TGACTTTCCC ATGATGGCTT  1800
TTGCTCTGGA ATGGACCCTT CTCCTTAGTA ACAGGCCTCT TACCACAATC TTCGTTTTTT  1860
TTTTTTTAAT GCTGTTTTCA AAAAGTGAGA GGCAGGTCCT CAACCACCCC CTGGAGCGCT  1920
CCAGAAGCCC AGGCGTGCCC TCATGCATTT CTCTGTGGTC TCTTGGCCCC CAGACCTCCT  1980
GTTTGATTGG ATAACTGCAT TTTTATACTG AGCACGTCTA AGTGGTCCTT TATTTTTTAT  2040
TTTCCCTATC GAGTGCTGTA GATGAAGAGT GATGACAATC CTGTAAATGT ACTAGAACTT  2100
TTTTATTAAA GGAACTTTTT CCCAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAC      2156
```

FIG. 2c.

FIG. 3a.

cDNA sequence for MDCK E-cadherin

```
CGGGCACCTG TGATTCGCGG AAGTCCTGCC GCCTCGCGCC GCCTCGCGCC CGGCTCTCGA   60
CCCCCGCCCG CCATGGGCCC TCGGTACGGC GGCGCCCCCG CGCTCCTGCT CCCGCTGCTG  120
CTGCTGCTGC AGGTCTCATC GGGGCTCTGC CAAGAGCCGG AGCCCTGCCG CCCTGGCTTT  180
GGCGCTGACA GCTACACGTT CACCGTGCCC CGGCGACACT TGGAGAGAGG CCGTGTCCTG  240
GGCAGGGTGA GTTTTGAAGG ATGCACCGGT CTACCTAGGA CAGCCTATGT TTCTGATGAC  300
ACCCGATTCA AAGTGGGCAC AGATGGTGTG ATTACAGTCA AGCGGCCTCT ACAACTTCAT  360
AAACCAGAGA TAAGTTTTCT TGTCCATGCC TGGGACTCCA GCCGCAGGAA GCTCTCCACC  420
AGAGTTAGGC TGAAGGCAGC GACGCACCAC CACCACCACC ATCATGATGC TCCCTCTAAA  480
ACCCAGACAG AGGTGCTCAC ATTTCCCAGT TCCCAGCATG GACTCAGAAG ACAGAAGAGA  540
GACTGGGTTA TCCCTCCTAT CAGCTGCCCG GAAAACGAGA AAGGCCCATT TCCTAAAAAC  600
CTGGTTCAGA TCAAGTCTAA CAGGGACAAA GAAATCAAGG TTTTCTACAG CATCACTGGC  660
CAAGGAGCTG ACGCACCTCC TGTTGGTGTG TTTATTATTG AAAGAGAAAC AGGATGGCTG  720
AAGGTGACTG AGCCTCTGGA TAGAGAACAA ATTGCTAAGT ACATTCTCTA CTCTCATGCC  780
GTATCTTCTA ATGGGAATGC GGTTGAAGAC CCAATGGAGA TCGTGATCAC GGTGACAGAT  840
```

FIG. 3b.

CAGAATGACA	ACAAGCCCGA	GTTCACCCAG	GCAGTCTTCC	AAGGATCTGT	CACGGAAGGT	900
GCCCTTCCAG	GCACCTCTGT	GATGCAGGTG	ACAGCCACAG	ATGCGGATGA	TGATGTGAAT	960
ACCTACAACG	CTGCCATCGC	TTACAGCATC	CTCACACAAG	ACCCCCTCCT	GCCTAGCAGC	1020
ATGATGTTCA	CTATCAACAA	GGACACAGGA	GTCATCAGCG	TGCTCACCAC	TGGGCTGGAC	1080
CGAGAGGGTG	TCCCCATGTA	CACCTTGGTG	GTTCAGGCTG	CTGACCTGCA	AGGCGAAGGC	1140
TTAACTACAA	CTGCAACAGC	TGTGATCACA	GTCACTGACA	TCAATGATAA	CCCCCCCATC	1200
TTCAACCCAA	CCACGTACCA	GGGACGGGTG	CCTGAGAACA	AGGCTAACGT	CGAAATCGCT	1260
GTACTCAAAG	TGACGGATGC	TGATGTCCCC	GATACCCCGG	CCTGGAGGGC	TGTGTACACC	1320
ATATTGAACA	ATAACAATGA	TCAATTTGTT	GTCACCACAG	ACCCAGTAAC	TAACGACGGC	1380
ATTTTGAAAA	CAACTAAGGG	CTTGGATTTT	GAGGACAAGC	AGCAGTATGT	CTTGTACGTG	1440
ACTGTGGTGA	ACGTGACCCC	GTTTGAGGTC	ATCCTCTCCA	CCTCCACAGC	CACTGTCACT	1500
GTGGACGTGG	AAGATGTGAA	TGAAGCCCCC	ATCTTCATCC	CTTGCCCAAA	GGTAGTGTCA	1560
ATCCCTGAAG	ACTTTGGTGT	GGGCCAGGAA	ATCACATCCT	ACACCGCCGA	GGATCCAGAT	1620
ACATATATGG	AACAGAGGAT	AACGTATCGG	ATTTGGAGGG	ATGCTGCCGG	TTGGCTGGAG	1680
GTTAATCCAG	AATCTGGTGC	CATTTTCACT	CGGGCTGAGC	TGGACAGAGA	GGATTTTGAG	1740

FIG. 3c.

```
CACGTGAAGA ATAGCACGTA TGAAGCCCTC ATTATAGCCA TTGACTTCGG TTCTCCAGTT  1800
GCTACTGGAA CGGGAACTCT TCTACTGGTC CTCTCTGATG TGAATGACAA TGGCCCCATT  1860
CCAGAACCTC GAAATATGGA CTTCTGCCAG AAAAACCCAC AGCCTCATGT CATCAACATC  1920
ATTGATCCAG ATCTTCCCCC CAACACATCT CCCTTCACAG CAGAACTAAC ACACGGCGCA  1980
AGTGTCAACT GGACCATCGA GTACAATGAC CCAGCTCGTG AATCTCTAAT TTTGAAGCCA  2040
AAGAAAACTT TAGAGTTGGG TGACTACAAA ATAAATCTCA AGCTCACAGA TAACCAGAAC  2100
AAGGACCAGG TGACCACCCT ATATGTGTTT GTGTGCGACT GCGAAGGTGT CGTCAACAGC  2160
TGCAAGAGGA CGGCGCCTTA CGCCGAAGCA GGCTTGCAGG TTCCTGCCAT CTTGGGCATT  2220
CTCGGAGGAA TCCTCGCTCT ACTAATCCTG ATTCTGCTGC TTCTGCTATT TGTTCGGAGG  2280
AGAAGGGTGG TCAAAGAGCC CTTACTTCCC CCAGAAGATG ACACCCGGGA CAATGTTTAT  2340
TACTATGATG AAGAAGGAGG TGGAGAGGAG GATCAGGACT TTGACTTGAG CCAGTTGCAC  2400
AGGGGCCTGG ATGCTCGGCC TGAAGTGACT CGCAATGATG TGGCCCCAAC CCTCCTGAGT  2460
GTGCCCCAGT ATCGGCCCCG CCCTGCCAAT CCTGATGAAA TTGGAAACTT TATTGATGAA  2520
AACCTGAAGG CAGCGGACAC TGACCCTACT GCTCCTCCTT ATGACTCTCT GCTCGTGTTT  2580
GACTATGAAG GAAGCGGTTC TGAAGCTGCT AGTCTGAGCT CCTTGAACTC CTCAGAGTCA  2640
GACCAAGACC AGGACTATGA CTACCTGAAT GAATGGGGCA ATCGCTTCAA GAAGCTGGCG  2700
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FIG. 3d.

```
GACATGTATG GAGGTGGCGA GGACGACTAG GGGACTTGAG ACAAATGAAG ATGAGTCCTT  2760
ATACCATGTG GTAGAAAATG CGGAGGTGAC TGTTTTCAGC TCCCTTCATC TGAGAGGAAT  2820
TTCTGGAGAA GAGAAAATGC ACAGTGATAT ATAGTTAGGA TAGTTAGGAT TTCTACTTTA  2880
TAGATCTAAT CTGTGTGTTT GTTAGAACGA TTTTGACCTA TTCTTTGAAG CTTTTTTTTC  2940
TTTCTTTCAT CATTCTTTAA ATGGTGATGC TGTCCAAAAG ACCCCCCACA TGTTTATATT  3000
TCAAAAGAAT AGCTAAAGCC TCCAGAAGGT TCTGCTAGCA ATTTCGAGAT TGCCTTATTG  3060
ACTTGTCTCA TTTTTTTAAA GGAAGGTAGG GCTAAACTAC CCTATTGTGT TTGTGTGTGT  3120
GTGTGTGTAT GTGTAATTAT TTTTAATTTG TGTTCTTTTT TCTCCTATCA CTGCACTGGT  3180
GTCCCGTGTT CTAATAACCA CTCTTAACTC CTTCTGAACT TACATTGCCT CAGACAGGAG  3240
TTCTCTGCTG CAGAAATTAT TGGGCCCTTT CAGGATAAGA GACTTGGTCT TAGTTTGATG  3300
GTAGTGTGAC TGGGTATTAT GGACTCGTAA GGACTTTAGT GGTTCTCCTT TTTTATTTCC  3360
TAAGTACATA AATTGAAATT CATATCCATC CACTGACTTG TTCTGCATTA AGTGTGTTTG  3420
TCATGTGGAC GTCATTATTG GGCTACTTTG GTTCTGAACA AGGAGCATTG ACCAGAAAAG  3480
GTGGTGAATT TTCAGGTGCC ACTCAACTTC TAATGTTCAC TTATCACTCA AACAGAAGAG  3540
TGATCTATTC TGACGTTTAG CGTAGTGCCT GCAGTGCTGC AGCCAAAGAT TGAAGGCGGA  3600
```

```
TTGTCAAAGC CAAGGGCAAC ATGAAAAATG GACTTGGAGG TGGCAGGCGG GATGGGTCAT  3660
TGAGCCTGGC GTTTTAGCAA ACTGATGCTG AGGATAACTG AGGTGGCTCT ACCTCTAGTC  3720
CTGAAAATTC TGAAGAATGG AAGAATCCCG ACAAGTGTGT CCTATCGCGA TCCTTAGGTC  3780
ACAGTTTGTA CCTGAGGCCA AGAATCCCCA GGTGCCTGCT TTTGTTAATG TCTACCGAAA  3840
ATGCAGCCTG ATCTGGACTC AGGTGCCCCA ATTCTAAGTG TGCATAGAAA ACTGACAATA  3900
TTAGGAAATT CTTTTTCCCC CCTTAGGAGC AGGAAGAAAA TATGACCCTA AAGGGTTTTG  3960
GCAAAGGGAA GGTGGGGAGA GCTTTGACTT GGATTTTTTT TAAATTGAAA TGTGAACTTC  4020
AAGGAACTTT TGACAACCAT GGGAAATAAT TTTATCTTAA ATTGCTTTAC TGTCTGTCAG  4080
CTGTTTTTCA AAGAAAAAAA AAATCATCCC TGCAATCACT TCTTGGAATT GTCTTGATTT  4140
TTCAGCAATT TAAACTCTAA TTTAGTCCTG TATAGAGAAT GTTAATGTAG TTTTGAGTGT  4200
ATATGTGTGT GGGTACGGAT AATTTTGTAT TTTCTTTAGG TCTGGAAAAG GAAAACAATT  4260
TAAGCTGCGA AAATTCTTAA ATATTCATTT TTATAAATTT TATTAAAGAA TTTTGTTAAA  4320
AAAAAAAAAA AAA                                                     4333
```

FIG.3e.

FIG. 4a.

N-cadherin restriction map

```
        BstBI
        AsuII
    EcoRI     XmnI
    |   |     |
GAATTCGAACCCCTTCGTTTCATTATGCAAGACTGGATTTCCTGAAGATGTGTACAGTGC   60

          SmaI
        XmaI
        AvaI
        | |
AGTCTTGTCCCGGGATGTGCTGGAAGGACAGCCCCTTCTCAATGTGAAGTTTAGCAACTG  120

CAATGGGAAAAGAAAAGTACAGTATGAGAGCAGCGAGCCAGCAGATTTTAAGGTGGATGA  180

                          HindIII
                          |
AGATGGCATGGTGTATGCCGTGAGAAGCTTCCCCCTCTCATCTGAACACTCGAAGTTCCT  240

GATATACGCTCAAGACAAAGAGACTCAGGAAAAGTGGCAAGTAGCAGTAAAACTGAGCCT  300

              SauI
              Eco81I
              Bsu36I
             EcoNI
             ||
CAAACCAGCCCTACCTGAGGATTCAGTGAAGGAATCACGAGAAATAGAAGAAATAGTGTT  360
```

FIG. 4b.

```
                                              BspMI
                                          PstI
                                          |  |
TCCAAGACAAGTGACTAAGCACAATGGCTACCTGCAGAGGCAGAAGAGAGACTGGGTTAT   420

                                                      SstI
                                                      SacI
                                      Eco0109         HgiAI
                                      EaeI            Bsp1286
                                      DraII           BanII
                                      |               |
CCCTCCCATCAACTTGCCAGAAAACTCCAGAGGGCCTTTTCCTCAAGAGCTCGTCAGGAT   480

  XhoII                                                    AlwNI
  |                                                        |
CAGATCCGATAGAGATAAAAACCTTTCTCTGCGGTACAGCGTAACTGGGCCAGGAGCTGA   540

                                                  PvuII
                                                  |
CCAGCCTCCAACTGGTATCTTCATTATCAACCCCATCTCAGGTCAGCTGTCAGTAACCAA   600

                                                   NspHI
    BstXI                                      Bsp1286          AseI
    |                                          |   |            |
```

FIG. 4c.

```
GCCTCTGGATCGTGAGCTGATAGCCCGGTTTCATTTGAGGGCACATGCAGTGGATATTAA   660

                              Tth111I
                              |
TGGAAACCAAGTGGAGAACCCCATCGACATTGTCATCAACGTTATTGACATGAATGATAA   720

                                             SauI
                                             Eco81I
                                             Bsu36I
                                            AlwNI
                                            ||
CAGACCTGAGTTCTTACACCAGGTTTGGAATGGGACAGTTCCTGAGGGATCAAAGCCGGG   780

    NdeI
    |
AACATATGTGATGACGGTCACTGCGATTGATGCTGACGATCCAAATGCCCTCAATGGGAT   840

                              HaeII
                              BbeI
                           NarI
                          BanI
                       EconI AhaII                     NspHI
                                                  AflIII
                       |    ||   |                     |   |
GTTGAGGTACAGAATCCTGTCCCAGGCGCCAAGCACCCCTTCGCCCAACATGTTTACAAT   900

                                   PvuII
                                   |
CAACAATGAGACTGGGGACATTATCACGGTGGCAGCTGGACTTGACAGAGAAAAGTACA    960
```

FIG. 4d.

```
     AccI                                                NdeI
     |                                                   |
ACAGTATACGTTAATAATTCAAGCTACAGACATGGAAGGCAATCCCACATATGGCCTTTC  1020

                                             HincII      BspMII
                                             |           AccIII
                                                         |
CAACACAGCCACGGCTGTCATCACGGTGACAGATGTCAACGACAATCCTCCGGAGTTTAC  1080
TGCCATGACGTTCTATGGTGAAGTCCCTGAAAACAGGGTAGATGTCATCGTCGCTAATCT  1140

                                       Cfr10I
                                       |
AACAGTGACAGATAAGGATCAGCCCCACACACCGGCCTGGAACGCCATCTACAGAATCAG  1200

                 NaeI
                 Eco52I
                 EagI
               Cfr10I
               |  |
CGGTGGAGACCCCGCCGGCCGCTTTGCCATTCAAACTGACCCCAACAGCAACGACGGTTT  1260
AGTCACCGTAGTAAAACCAATCGACTTTGAAACAAATAGGATGTATGTCCTTACTGTCGC  1320

    PstI                 StyI                           HincII
    |                    |                              |
TGCAGAAAATCAAGTGCCATTAGCCAAGGGTATTCAGCATCCACCTCAGTCAACTGCGAC  1380
```

FIG. 4e.

```
Tth111I                          ClaI
|                                |
TGTGTCTGTCACAGTTATCGATGTGAATGAAAATCCTTATTTTGCCCCAAATCCAAAGAT  1440

                                 XmnI         BanI
                                 StuI         Asp718      HpaI                      Eco0109
                               EaeI      Cfr10I KpnI      HincII                    DraII
                               |  |      |   |    |       |                         |
CATTCGCCAAGAAGAAGGCCTTCACGCCGGTACCGTGTTAACAACGTTTACTGCTCAGGA  1500

        ClaI
        |
CCCAGATCGATATATGCAGCAAAATATCAGATACACCAAATTATCCGATCCTGCAAACTG  1560

GCTAAAAATAGACTCTGTGAATGGGCAGATAACTACCATTGCTGTTTTGGACAGAGAATC  1620

ACCGAATGTGAAAGCCAATATATACAATGCTACTTTCCTTGCTTCTGACAATGGAATCCC  1680

                           XhoII
                           PstI
                           BglII                  AseI
                           |                      |
TCCTATGAGTGGAACGGGAACACTGCAGATCTATTTACTTGATATTAATGACAATGCCCC  1740

                                         BspMII
                                         AccIII
                                         |
TCAAGTGTTACCTCAAGAGGCAGAGATTTGTGAAACTCCGGACCCCAATTCAATTAACAT  1800
```

FIG. 4f.

```
                                   PflMI
                                   |
CACAGCACTTGATTATGACATTGATCCAAATGCTGGACCATTTGCTTTTGATCTTCCTTT  1860

                                                         CelII
                                                         |
GTCTCCAGTGACTATTAAGAGAAATTGGACCATCACTCGGCTTAATGGTGATTTTGCTCA  1920

                                    XhoII
                                    |
GCTTAACTTAAAGATAAAATTTCTTGAGGCCGGGATCTACGAAGTTCCAATCATAATCAC  1980

AGATTCGGGTAATCCTCCCAAATCGAATATCTCCATCCTTCGGGTGAAGGTTTGCCAGTG  2040

                                                        Cfr10I
                                                        Bsp1286
                                                     BanI  BanI
                                                     |  |  |
TGATTCCAACGGGGACTGCACAGATGTGGATCGAATTGTGGAGCAGGGCTGGGCACCGG  2100

   HaeII
   BbeI
NarI
AhaII
|  |
```

FIG. 4g.

```
CGCCATCATCGCCATCCTGCTTTGCATCATCATCCTGCTCATTCTCGTTCTGATGTTCGT   2160

GGTATGGATGAAACGCCGGGATAAAGAACGCCAGGCCAAACAACTTTTAATTGATCCAGA   2220

                        DraI
                   SspI AhaIII
                   |    |
AGATGATGTAAGAGATAATATTTTAAAATATGATGAAGAAGGTGGAGGAGAAGAAGACCA   2280

GGACTACGATTTGAGCCAGCTCCAGCAGCCTGATACGGTAGAGCCAGATGCCATCAAGCC   2340

                                             Bsp1286
                          EaeI               BanII
                          |                  |
AGTTGGAATCCGACGGTTGGATGAGAGGCCCATCCATGCGGAGCCCCAGTACCCGGTTCG   2400

                                              Eco0109
                                              EaeI
       PstI                               AseI DraII
       |                                  |    |
ATCTGCAGCCCCACACCCAGGGGACATCGGGACTTCATTAATGAGGGCCTTAAAGCTGC   2460

TGACAACGATCCCACCGCTCCGCCCTACGACTCCCTCTTAGTCTTTGACTATGAAGGCAG   2520

                          SstI
                          SacI
               Eco0109    HgiAI
         Eco52I           Bsp1286
         EagI  DraII      BanII
         |     |          |
TGGCTCCACGGCCGGGTCCTTGAGCTCCCTTAATTCCTCCAGTAGTGGAGGTGAGCAGGA   2580
```

FIG. 4h.

```
                                   Bsp1286
                                   BanII
                                   ApaI
                                Eco0109
                                DraII
                               Eco0109
                               EaeI
                               DraII                                 NspHI
                               || |                          AflIII
                               || |                          |   |
CTATGACTATCTGAACGACTGGGGGCCCCGCTTCAAGAAACTCGCTGACATGTACGGTGG  2640

AGGTGATGACTGAACTTCAGGGTGAACTTGGTTTTTGGACAAGTACAAACAATTGCAACT  2700

                BsmI                          AccI
                |                              |
GATATTCCCAAAAAGCATTCAGAAGCTAGGCTTTAACTTTGTAGTCTACTAGCACAGTGC  2760

                                           Bsp1286
                       AlwNI               BanII
                       |                   |
TTGCTGGAGGCTTTGGCAGAGGCTGCAAACCAATTTGGGCTCAGAGGAATATCGGTGAT  2820
                               SstI
                               SacI
                               HgiAI
                               Bsp1286
```

FIG.4i.

```
                        BanII
                        |
CCAATACTGTTTGGAAAACACTGAGCTCAGTTACACTTGAATTTTACAGTACAGAAGCAC  2880
TGGGATTTTATGTGCCTTTTTGTACCTTTTTCAGATTGGAATTAGTTTTATGTTTAAGGC  2940

                                        SspI
                                        |
TTTAATGGTACTGATTTCTGAAATGATAAGTAAAAGACAAAATATTTTGTGGTGGGAGCA  3000
GTAAGTTAAACCATGATATGCTTCGACACGCTTTTGTTACATCGCATTTGCTTTTATTAA  3060

                                                       StyI
                                                       |
AAATATGGAATTAAACAGACAAACCAACCACTCATGGAGCAATTTTATTACCTTGGGGGC  3120

            BstXI
            |
TGAGACCATGAGATTGGAAAATGTACATTATTTCTAGTTTTAGACTTTAGTTTCTTGTTT  3180

                                  PvuII
                                  |
TGTTTTTTTTTTCCACTAAAATCTTAAAACTTACGCAGCTGGTTGCAAATAAAGGGAGTT  3240

                             XmnI
                             |
TTCATATCACCAATTTGTAGCAAAATTGAATTTTTTCATAAACTAGAATGTTAGACACAT  3300
TTTGGTCTTAATCCATGTACACTTTTTTATTTACTGTATTTTTTCCACTTCACTGTAAAA  3360
ATGGTATGTGTACATAATGTTTTATTGGCATAGTCTATGGAGAAGTGCAGAAACTTCAGA  3420
```

FIG.4j.

```
    NspHI
 AflIII                                 NspHI
 |   |                                        |
ACATGTGTATGTATTATTTGGACTATGGATTCAGGTTTTTTGCATGTTTATATCTTTCGT  3480

TATGGATAAAGTATTTACAAAACAAAGTGACATTTGATTCAATTGTTGAGCTGTAGTTAG  3540

AATACTCAATTTTTAATTTTTTAATTTTTTTTATTTTTTATTTTCTCTTTTTGTTTGGGG  3600

AGGGAGAAAAGTTCTTAGCACAAATGTTTTACATAATTTGTACCAAAAAAAAACAAAAAA  3660

                          BstEII                      PstI
                         |                            |
AAAGGAAAGACAAGAAATGAAAGGGGTGACCTGACACTGGTGGTACTACTGCAGTGTGTG  3720

                                  DraI
     DraI                         AhaIII
     AhaIII                 HindIII
     |                     |      |
TTTTTAAAAAAAAATGAAAAAAAAAAAAGCTTTTAAACTGGAGAGACTTCTGACAACAGCT  3780

TTGCCTCTGTATTGTGTACCAGAATATAAATGATACACCTCTGACCCCAGCGTTCTGAAT  3840

AAAATGCTAATTTTGGAAAAAAAAAAAAAAAAAAA                           3875
```

P-cadherin restriction map

FIG. 4k.

```
     BstBI
     AsuII
 EcoRI     XmnI                         DraIII   AlwNI     AlwNI
 |    |     |                            |        |         |
GAATTCGAACCCCTTCGCTGAGAACACAGTGAGCCACGAGGTGCAGAGGCTGACAGTGAC   60

         AhaII
         |
TGATCTGGACGCCCCTAACTCACCAGCATGGCGTGCCACCTACCGCATCGTGGGAGGTGA  120

                                AvaI
                                |
CAACGGGGACCATTTTACCATCACTACTGACCCCGAGAGCAACCAGGGTATCCTGACCAC  180

                                                      BclI
                                                      |
CCAGAAGGGCTTGGATTTTGAGGCCAAAACCCAGCACACCCTGTACGTCGAAGTGATCAA  240

                                              BstXI     EcoNI
                                              |         |
CGAGGTTCCCTTTGTGGTGAAACTCCCGACCTCCACAGCCACCGTAGTGGTCCTCGTGGA  300

GGATGTGAATGAGCCACCCGTGTTTGTCCCCCCGTCCAAAGTCATCGAAATCCAGGAGGG  360

                                        Eco0109
                                        DraII
                                        |
CATCTCCACTGGGGAGCCTATTTGTGCCTACACTGCACGGGACCCAGACAAGGGGAGTCA  420
```

FIG. 41.

```
                                        NheI
                                     BstXI                 PflMI
GAAGATCAGTTACCACATCCTGAGAGACCCAGCAGGGTGGCTAGCGATGGACCCAGACAG   480

TGGACAAGTCACTGCCGCAGGGGTCTTGGACCGTGAGGATGAGCAGTTTGTGAGAAACAA   540

                                         Bsp1286
                   BalI                  BanII
CATCTACGAAGTCATGGTCTTGGCCACAGATGATGGAGCCCTCCCACCACTGGCACAGG   600

EcoO109                                                 Bsp1286
DraII                                             AvaI  BanII
GACCCTCCTGCTAACACTGATGGACATCAATGACCACGGTCCGGTCCCCGAGCCCCGTCA   660

                         Bsp1286
GATCACCATCTGCAACCAAAGCCCTGTGCCCCAGGTGCTAAACATCACAGACAAGGACTT   720

                                                     AatII
                    EaeI                          AhaII
GTCCCCCCACACTGCCCCTTTCCAGGCCCAACTCACACATGACTCGGACGTCTATTGGAC   780

         HincII                                   XmnI
AGCAGAAGTCAACGAGAAAGGAGACGCAGTAGCCTTGTCCCTGAAGAAGTTCCTAAAGCA   840
```

FIG.4m.

```
                 HgiAI
                 Bsp1286
          ApaL1                                       PvuII
AGGCGAATACGATGTGCACCTTTCCCTGTCCGACCACGGCAACAAGGAACAGCTGACAGT   900

                                                     BspMI
                                                     Eco0109
BclI         DraIII                   BstEII         DraII
GATCAGAGCCACCGTGTGTGACTGCCACGGCAACATGGTGACCTGCCGGGACCCCTGGAC   960

GTGGGGTTTCCTCCTCCCCATCCTGGGTGCTGCCCTGGCTCTGCTGCTCCTTCTGCTGGT  1020

  HgiAI
  Bsp1286                                XmnI
GCTCCTATTCTTGGTGAGAAAGAAACGGAAGATCAAGGAACCCCTTCTCCTCCCAGAAGA  1080

             Tth111I
TGATACCCGTGACAACGTCTTCTACTACGGCGAAGAGGGGGGTGGCGAGGAGGACCAGGA  1140

                                        SauI
                                        Eco81I
                                 EaeI   Bsu36I
CTATGACATCACCCAGCTCCACCGGGGTCTGGAGGCCCGGCCTGAGGTGGTTCTCCGCAA  1200
```

FIG. 4n.

```
      BanI
       |
CGATGTGGCACCATCCTTCATCCCCACACCCATGTACCGTCCTCGGCCAGCCAACCCAGA  1260

TGAAATCGGCAACTTCATCATTGAGAACCTGAAGGCAGCCAACACAGACCCCACGGCCCC  1320

GCCCTACGACTCCCTGTTGGTGTTCGACTATGAGGGCAGTGGCTCCGATGCCGCCTCTCT  1380

     SstI
     SacI
     HgiAI
     Bsp1286
     BanII
     |
GAGCTCGCTCACCTCCTCAACCTCTGACCAGGACCAAGACTACAACTATCTGAATGAGTG  1440

                              NspHI
                          AflIII
                          |   |
GGGCAGCCGCTTCAAGAAGCTGGCGGACATGTACGGCGGGGGCCAGGACGACTAGGACTC  1500

                                                        StyI
                PstI      StyI                         BalI
                |         |                            ||
CCTAAACGCCGGGCTGCAGCAGCGTCTCCAAGGGGTCACTATCCCCACGTTGGCCAAGGA  1560

                                                 StuI
                                               EaeI
                                               | |
CTTTGCAGCTTGTTGAGAATTGGCCTTAGCAACTTGGAGGGAAGAGGCCTCGAAACTGAC  1620
```

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FIG. 4o.

```
 BspMI
 |
CTCAAAGGGGCAGGTCTCTATGCCTTTCAGAACGGAGGAACGTGGCAGTTTGATTTCAA  1680

         HgiAI
         Bsp1286      EcoNI
         |            |
CAGTGAGCACCTCTTAGCCTAAGCCAGGGCTGCTCAATTTCTGGAGTCTCCTCGCTACC  1740

                           Eco0109
                           DraII
                      Eco47III
         CelII       HaeII
         |    |  |  |
ATAAAATGCTCAGCGCTGGGTCCTGGGTTTTGACTGACTCTGACTTTCCCATGATGGCTT  1800

                                            StuI
                                          EaeI
                                          |  |
TTGCTCTGGAATGGACCCTTCTCCTTAGTAACAGGCCTCTTACCACAATCTTCGTTTTTT  1860

                                       Eco0109                 HaeII
                         BspMI         DraII           PflMI   Eco47III
                         |             |               |       |  |
TTTTTTTAATGCTGTTTTCAAAAAGTGAGAGGCAGGTCCTCAACCACCCCCTGGAGCGCT  1920

                    Bsp1286 NsiI
                    |        |
CCAGAAGCCCAGGCGTGCCCTCATGCATTTCTCTGTGGTCTCTTGGCCCCCAGACCTCCT  1980
```

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FIG. 4p.

```
                                    HgiAI
                                    Bsp1286
                                    |
GTTTGATTGGATAACTGCATTTTTATACTGAGCACGTCTAAGTGGTCCTTTATTTTTTAT  2040

TTTCCCTATCGAGTGCTGTAGATGAAGAGTGATGACAATCCTGTAAATGTACTAGAACTT  2100

                 XmnI
                 |
TTTATTAAAGGAACTTTTTCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAC      2156


      E-cadherin restriction map

   BanI
   |
CGGGCACCTGTGATTCGCGGAAGTCCTGCCGCCTCGCGCCGCCTCGCGCCCGGCTCTCGA    60

                        BanII            HaeII
                        ApaI             BbeI
                    EaeI              NarI
                StyI EcoO109          AhaII
                NcoI DraII           BanI
                |   || |             || |
CCCCCGCCCGCCATGGGCCCTCGGTACGGCGGCGCCCCCGCTCCTGCTCCCGCTGCTG    120

BspMI      PstI           BanII            BanII    BglI
|          |              |                |        |
CTGCTGCTGCAGGTCTCATCGGGGCTCTGCCAAGAGCCGGAGCCCTGCCGCCCTGGCTTT    180
```

FIG. 4q.

```
     HaeII      AflIII
     |          |
GGCGCTGACAGCTACACGTTCACCGTGCCCCGGCGACACTTGGAGAGAGGCCGTGTCCTG   240

                                StyI
                            AccI
                       Cfr10I   AvrII
                       |    |   |
GGCAGGGTGAGTTTTGAAGGATGCACCGGTCTACCTAGGACAGCCTATGTTTCTGATGAC   300

ACCCGATTCAAAGTGGGCACAGATGGTGTGATTACAGTCAAGCGGCCTCTACAACTTCAT   360

AAACCAGAGATAAGTTTTCTTGTCCATGCCTGGGACTCCAGCCGCAGGAAGCTCTCCACC   420

                                         BspHI
                                         |
AGAGTTAGGCTGAAGGCAGCGACGCACCACCACCACCATCATGATGCTCCCTCTAAA   480

                HgiAI
                |
ACCCAGACAGAGGTGCTCACATTTCCCAGTTCCCAGCATGGACTCAGAAGACAGAAGAGA   540

                         PvuII                EaeI
                         |                    |
GACTGGGTTATCCCTCCTATCAGCTGCCCGGAAAACGAGAAAGGCCCATTTCCTAAAAAC   600

                                                          BalI
                                                          |
CTGGTTCAGATCAAGTCTAACAGGGACAAAGAAATCAAGGTTTTCTACAGCATCACTGGC   660
```

FIG. 4r.

```
StyI
|
CAAGGAGCTGACGCACCTCCTGTTGGTGTGTTTATTATTGAAAGAGAAACAGGATGGCTG   720

AAGGTGACTGAGCCTCTGGATAGAGAACAAATTGCTAAGTACATTCTCTACTCTCATGCC   780

                BsmI                BclI
                |                   |
GTATCTTCTAATGGGAATGCGGTTGAAGACCCAATGGAGATCGTGATCACGGTGACAGAT   840

                                        XhoII
            AvaI                    StyI                BanI
            |                       |   |               |
CAGAATGACAACAAGCCCGAGTTCACCCAGGCAGTCTTCCAAGGATCTGTCACGGAAGGT   900

         BanI BspMI
         |    |
GCCCTTCCAGGCACCTCTGTGATGCAGGTGACAGCCACAGATGCGGATGATGATGTGAAT   960
ACCTACAACGCTGCCATCGCTTACAGCATCCTCACACAAGACCCCCTCCTGCCTAGCAGC  1020

                                         HgiAI    BstXI
                                         |        |
ATGATGTTCACTATCAACAAGGACACAGGAGTCATCAGCGTGCTCACCACTGGGCTGGAC  1080

                   StyI                             BspMI
                   |                                |
CGAGAGGGTGTCCCCATGTACACCTTGGTGGTTCAGGCTGCTGACCTGCAAGGCGAAGGC  1140
```

FIG. 4s.

```
                              BclI
                        PvuII     AlwNI
TTAACTACAACTGCAACAGCTGTGATCACAGTCACTGACATCAATGATAACCCCCCCATC  1200

                                  BanI
TTCAACCCAACCACGTACCAGGGACGGGTGCCTGAGAACAAGGCTAACGTCGAAATCGCT  1260

                                                  BglI
GTACTCAAAGTGACGGATGCTGATGTCCCCGATACCCCGGCCTGGAGGGCTGTGTACACC  1320

                   BclI
ATATTGAACAATAACAATGATCAATTTGTTGTCACCACAGACCCAGTAACTAACGACGGC  1380
ATTTTGAAAACAACTAAGGGCTTGGATTTTGAGGACAAGCAGCAGTATGTCTTGTACGTG  1440

                                                       AlwNI
ACTGTGGTGAACGTGACCCCGTTTGAGGTCATCCTCTCCACCTCCACAGCCACTGTCACT  1500
GTGGACGTGGAAGATGTGAATGAAGCCCCCATCTTCATCCCTTGCCCAAAGGTAGTGTCA  1560

                                                       XhoII
                                                       BamHI
ATCCCTGAAGACTTTGGTGTGGGCCAGGAAATCACATCCTACACCGCCGAGGATCCAGAT  1620
                                                  Cfr10I
```

FIG. 4t.

```
ACATATATGGAACAGAGGATAACGTATCGGATTTGGAGGGATGCTGCCGGTTGGCTGGAG  1680

                                 BanI
                              PflMI
                              AlwNI               AvaI   CelII
                              |   |               |      |
GTTAATCCAGAATCTGGTGCCATTTTCACTCGGGCTGAGCTGGACAGAGAGGATTTTGAG  1740

  HgiAI
  |
CACGTGAAGAATAGCACGTATGAAGCCCTCATTATAGCCATTGACTTCGGTTCTCCAGTT  1800

GCTACTGGAACGGGAACTCTTCTACTGGTCCTCTCTGATGTGAATGACAATGGCCCCATT  1860

CCAGAACCTCGAAATATGGACTTCTGCCAGAAAAACCCACAGCCTCATGTCATCAACATC  1920

          XhoII
          BglII
          |
ATTGATCCAGATCTTCCCCCCAACACATCTCCCTTCACAGCAGAACTAACACACGGCGCA  1980

      HincII
      |
AGTGTCAACTGGACCATCGAGTACAATGACCCAGCTCGTGAATCTCTAATTTTGAAGCCA  2040

AAGAAAACTTTAGAGTTGGGTGACTACAAAATAAATCTCAAGCTCACAGATAACCAGAAC  2100

                                                         PvuII
          BstEII                                    HincII
          |                                         |    |
AAGGACCAGGTGACCACCCTATATGTGTTTGTGTGCGACTGCGAAGGTGTCGTCAACAGC  2160
```

FIG. 4u.

```
                  HaeII
                  BbeI
              NarI
              AhaII
             BanI            BspMI                         BsmI
             | |  |             |                            |
TGCAAGAGGACGGCGCCTTACGCCGAAGCAGGCTTGCAGGTTCCTGCCATCTTGGGCATT  2220

CTCGGAGGAATCCTCGCTCTACTAATCCTGATTCTGCTGCTTCTGCTATTTGTTCGGAGG  2280

                                                SmaI
                                              XmaI
                   BanII                      AvaI
                     |                        | |
AGAAGGGTGGTCAAAGAGCCCTTACTTCCCCCAGAAGATGACACCCGGGACAATGTTTAT  2340

TACTATGATGAAGAAGGAGGTGGAGAGGAGGATCAGGACTTTGACTTGAGCCAGTTGCAC  2400

   Eco0109
   EaeI
   DraII
   |
AGGGGCCTGGATGCTCGGCCTGAAGTGACTCGCAATGATGTGGCCCCAACCCTCCTGAGT  2460

GTGCCCCAGTATCGGCCCCGCCCTGCCAATCCTGATGAAATTGGAAACTTTATTGATGAA  2520

AACCTGAAGGCAGCGGACACTGACCCTACTGCTCCTCCTTATGACTCTCTGCTCGTGTTT  2580
```

FIG. 4v.

```
               XmnI                     SstI
                                        SacI
                                        HgiAI
                                        BanII
               |                        |
GACTATGAAGGAAGCGGTTCTGAAGCTGCTAGTCTGAGCTCCTTGAACTCCTCAGAGTCA  2640

GACCAAGACCAGGACTATGACTACCTGAATGAATGGGGCAATCGCTTCAAGAAGCTGGCG  2700

      NspHI
  AflIII
  |   |
GACATGTATGGAGGTGGCGAGGACGACTAGGGGACTTGAGACAAATGAAGATGAGTCCTT  2760

ATACCATGTGGTAGAAAATGCGGAGGTGACTGTTTTCAGCTCCCTTCATCTGAGAGGAAT  2820

TTCTGGAGAAGAGAAAATGCACAGTGATATATAGTTAGGATAGTTAGGATTTCTACTTTA  2880

  XhoII
  BglII                                         HindIII
  |                                             |
TAGATCTAATCTGTGTGTTTGTTAGAACGATTTTGACCTATTCTTTGAAGCTTTTTTTTC  2940

                  DraI                              NspHI
                  AhaIII                        AflIII
                  |                             |   |
TTTCTTTCATCATTCTTTAAATGGTGATGCTGTCCAAAAGACCCCCCACATGTTTATATT  3000

                       EcoNI      NheI
                       |          |
TCAAAAGAATAGCTAAAGCCTCCAGAAGGTTCTGCTAGCAATTTCGAGATTGCCTTATTG  3060
```

FIG.4w.

```
                 DraI
                 AhaIII
                 |
ACTTGTCTCATTTTTTTAAAGGAAGGTAGGGCTAAACTACCCTATTGTGTTTGTGTGTGT  3120

GTGTGTGTATGTGTAATTATTTTTAATTTGTGTTCTTTTTTCTCCTATCACTGCACTGGT  3180

                                                  EcoNI
                                                  |
GTCCCGTGTTCTAATAACCACTCTTAACTCCTTCTGAACTTACATTGCCTCAGACAGGAG  3240

                                BanII
                                ApaI
                             Eco0109
                             DraII
            PstI            EaeI                Tth111I
            |           | |  |                  |
TTCTCTGCTGCAGAAATTATTGGGCCCTTTCAGGATAAGAGACTTGGTCTTAGTTTGATG  3300

GTAGTGTGACTGGGTATTATGGACTCGTAAGGACTTTAGTGGTTCTCCTTTTTTATTTCC  3360

TAAGTACATAAATTGAAATTCATATCCATCCACTGACTTGTTCTGCATTAAGTGTGTTTG  3420

             AatII
         AhaII
         |  |
TCATGTGGACGTCATTATTGGGCTACTTTGGTTCTGAACAAGGAGCATTGACCAGAAAAG  3480

              BanI
              |
GTGGTGAATTTTCAGGTGCCACTCAACTTCTAATGTTCACTTATCACTCAAACAGAAGAG  3540
```

FIG. 4x.

```
                                   PstI     PstI
                                   |        |
TGATCTATTCTGACGTTTAGCGTAGTGCCTGCAGTGCTGCAGCCAAAGATTGAAGGCGGA  3600

           StyI
           |
TTGTCAAAGCCAAGGGCAACATGAAAAATGGACTTGGAGGTGGCAGGCGGGATGGGTCAT  3660

TGAGCCTGGCGTTTTAGCAAACTGATGCTGAGGATAACTGAGGTGGCTCTACCTCTAGTC  3720

                                                      SauI
                                                      Eco81I
                                                NruI  Bsu36I
                                                |     |
CTGAAAATTCTGAAGAATGGAAGAATCCCGACAAGTGTGTCCTATCGCGATCCTTAGGTC  3780

             SauI
             Eco81I
             Bsu36I             BanI                AccI
             |                  |                   |
ACAGTTTGTACCTGAGGCCAAGAATCCCCAGGTGCCTGCTTTTGTTAATGTCTACCGAAA  3840

                         BanI                                SspI
                         |                                   |
ATGCAGCCTGATCTGGACTCAGGTGCCCCAATTCTAAGTGTGCATAGAAAACTGACAATA  3900

                         SauI
                         Eco81I
                         Bsu36I
                         |
TTAGGAAATTCTTTTTCCCCCCTTAGGAGCAGGAAGAAAATATGACCCTAAAGGGTTTTG  3960
```

```
                                                DraI
                                                AhaIII
                                                |
GCAAAGGGAAGGTGGGGAGAGCTTTGACTTGGATTTTTTTTAAATTGAAATGTGAACTTC  4020

                StyI
                NcoI
                |
AAGGAACTTTTGACAACCATGGGAAATAATTTTATCTTAAATTGCTTTACTGTCTGTCAG  4080

PvuII
|
CTGTTTTTCAAAGAAAAAAAAAATCATCCCTGCAATCACTTCTTGGAATTGTCTTGATTT  4140

          DraI
          AhaIII
          |
TTCAGCAATTTAAACTCTAATTTAGTCCTGTATAGAGAATGTTAATGTAGTTTTGAGTGT  4200

ATATGTGTGTGGGTACGGATAATTTTGTATTTTCTTTAGGTCTGGAAAAGGAAAACAATT  4260

                      SspI
                      |
TAAGCTGCGAAAATTCTTAAATATTCATTTTTATAAATTTTATTAAAGAATTTTGTTAAA  4320

AAAAAAAAAAAAA                                                 4333
```

FIG. 4y.

FIG. 5.

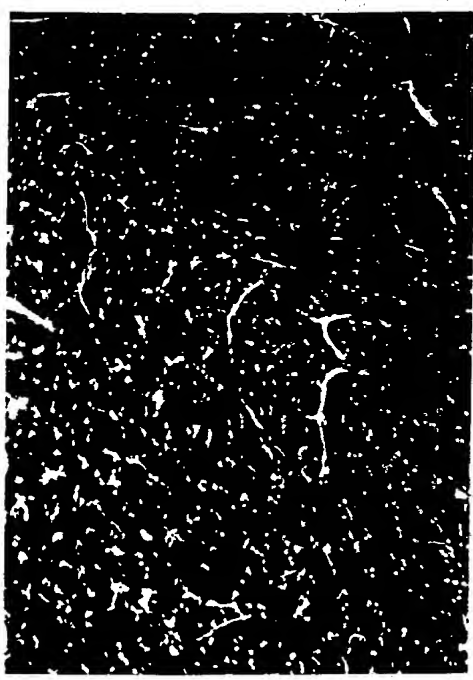

FIG. 6.

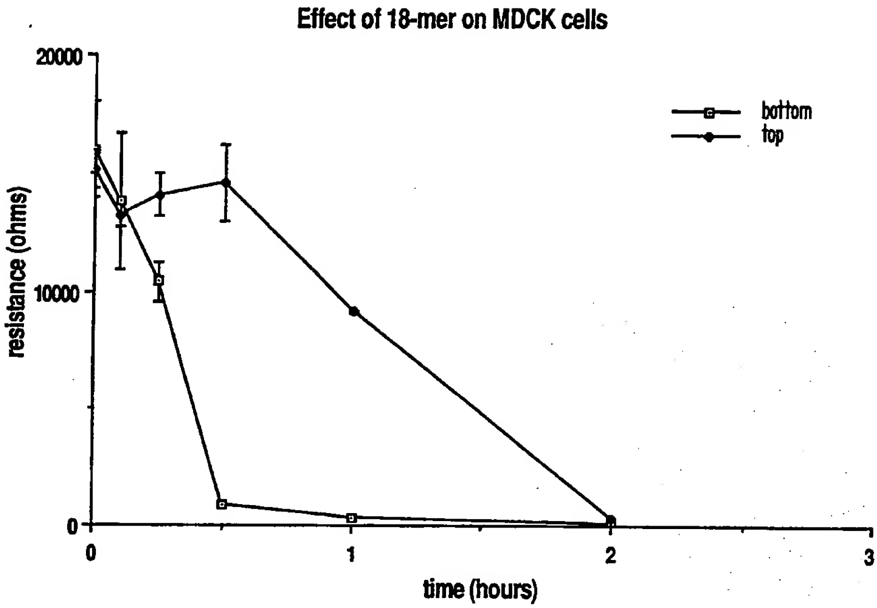
Effect of 18-mer on MDCK cells
20000
10000
0
resistance (ohms)
0
1
2
3
time (hours)
bottom
top

FIG. 7.

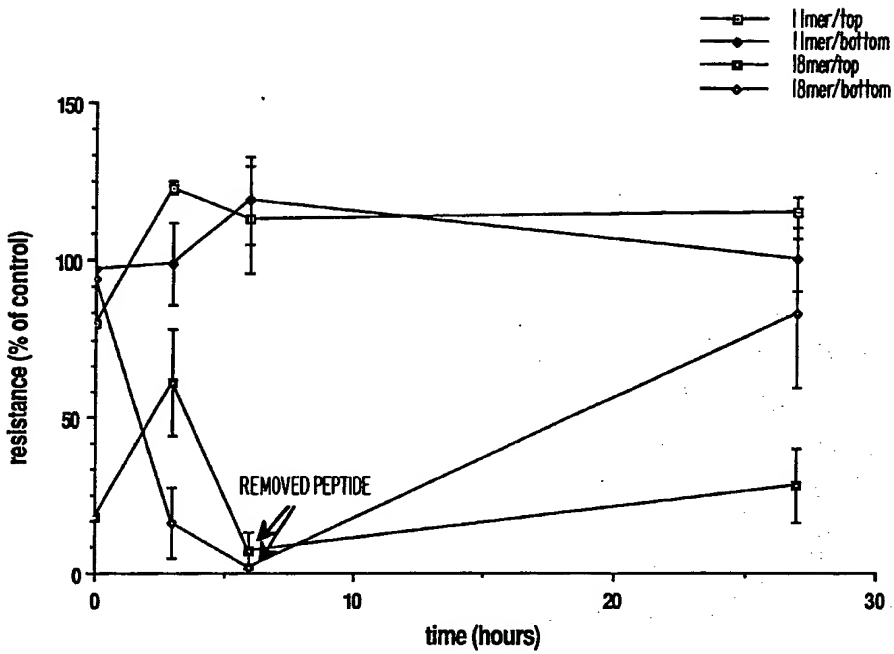
11mer/top
11mer/bottom
18mer/top
18mer/bottom
150
100
50
0
resistance (% of control)
REMOVED PEPTIDE
0
10
20
30
time (hours)

FIG. 8.

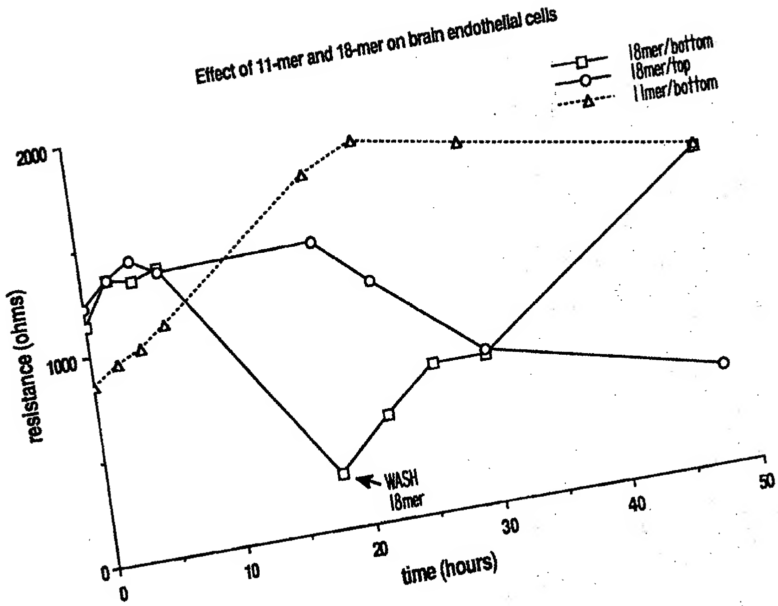
Effect of 11-mer and 18-mer on brain endothelial cells
18mer/bottom
18mer/top
11mer/bottom
2000
1000
0
resistance (ohms)
WASH
18mer
0
10
20
30
40
50
time (hours)

FIG. 9.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05105

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02, 39/00; C07K 7/08. 7/10, 13/00, 15/00, 15/28

U.S.Cl.: 530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387; 514/12, 13; 424/85.8, 85.91

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S. Cl.	530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387 514/12, 13 424/85.8, 85.91

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[5]

Data bases: Dialog (Files; Medline, Biosis, Chemical Abstracts, World Patents Index) Automated Patent Searching (1975-1990)

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
X Y	The EMBO Journal, Volume 4, No. 13A, issued December 1985, Vestweben et al., "Identification of a Putative Cell Adhesion Domain of Uvomorulin," pp. 3393-3398. See the Abstract and Discussion.	1-6,14-21,23-27 & 35-42 1-65
Y	Development, Volume 102, issued April 1988, M. Takeichi, "The Cadherins: Cell-cell Adhesion Molecules controlling Animal Morphogenesis," pp. 639-655 see the Summary and pages 643, 645 and 651.	1-65
X Y	The Journal of Cell Biology, Volume 107, issued October 1988, B. Gumbiner et al., "The Role of the Cell Adhesion Molecule Uvomorulin in the Formation and Maintenance of the Epithelial Junctional Complex," pp. 1575-1587 see the Abstract.	1-6,14-21,23-27, 35-42 1-6,14-27,35-47, 55-65

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
21 November 1990	04 FEB 1991
International Searching Authority[1]	**Signature of Authorized Officer**[20]
ISA/US	R. Keith Baker R. Keith Baker, Ph.D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	The EMBO Journal, Volume 6, No. 12, issued 1987, M. Ringwald et al., "The Structure of Cell Adhesion Molecule Uvomorulin Insights into the Molecular Mechanism of Ca^{++}-dependent Cell Adhesion," pp3347-3353, see pages 3647-3648.	1-13,22-34,43-54 and 63-65
Y	US, A, 4.671,958 (Rodwell et al.) 09 June 1987, see the Abstract and Column 7.	43-47 and 55-65
Y,P	Development Biology, Volume 139, No. 1, issued May 1990, O.W. Blaschuk et al., "Identification of a Cadherin Cell Adhesion Recognition Sequence," pp227-229, see the entire Document.	1-65

International Application No. PCT/US90/05105

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers__________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. X OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone Practice
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Attachment To PCT/ISA/210
Observations Where Unity Of Invention Is Lacking

Group I, claims 1-13 and 22-34, drawn to a composition for opening tight junctions and a method of use, classified in classes 530 and 514, subclasses 324, 326, 350 and 12 and 13, respectively.

Group II, claims 14-21 + 35-42, drawn to antibodies for opening tight junctions and methods of use, classified in classes 530 and 424, subclasses 387 and 85.8, respectively.

Group III, claims 43-54 and 63-65, drawn to a conjugates of a drug and a cell adhesion inhibitor, classified in class 530, subclasses 402, 409, and 345.

Group IV, claims 55-62, drawn to a conjugate of a drug and an antibody, classified in classes 530 and 424, subclasses 389, 390, 391 and 85.91, respectively.

Attachment To PCT/ISA/210
Detailed Reasons For Holding Lack Of Unity Of Invention:

PCT Rule 13.2 permits claims to "a" (one) product, "a" (one) method of making and "a" (one) method of using said product. The invention as set forth in Group I constitutes a combination of a product and a method of use. Groups II, III and IV one drawn to products that are distinct from that of Group I. Each of the products have a different structure and are distinct compositions as evidenced by their separate classification.